

CLAIMS AMENDMENTS:

Please cancel claims 60 and 68, without prejudice.

Please amend the claims as follows:

Claims 1-14 (Canceled)

15. (Withdrawn) An oligonucleotide primer for amplifying human genomic DNA encoding an ob polypeptide.

16. (Withdrawn) The oligonucleotide of Claim 15, which is selected from the group consisting of

HOB 1gF	5'-CCCAAGAAGCCCATCCTG-3' (SEQ ID NO:26)
HOB 1gR	5'-GACTATCTGGGTCCAGTGCC-3' (SEQ ID NO:27)
HOB 2gF	5'-CCACATGCTGAGCACTTGTT-3' (SEQ ID NO:28)
HOB 2gR	5'-CTTCAATCCTGGAGATACCTGG-3' (SEQ ID NO:29).

17. (Withdrawn) An ob polypeptide, which polypeptide is encoded by the DNA molecule of Claim 1.

18. (Withdrawn) An ob polypeptide, which polypeptide is characterized by having about 145 to about 167 amino acid residues, being expressed predominantly by adipocytes, and being capable of inducing a reduction of body weight in an animal.

19. (Withdrawn) The ob polypeptide of Claim 18 which has the amino acid sequence shown in Figure 1 (SEQ ID NO:2) or Figure 5 (SEQ ID NO:5).

20. (Withdrawn) The ob polypeptide of Claim 19 which has the amino acid sequence shown in Figure 3 (SEQ ID NO:4) or Figure 6 (SEQ ID NO:6).

21. (Withdrawn) An immunogenic fragment of an ob polypeptide, which polypeptide is

characterized by having about 160 amino acid residues, being expressed predominantly by adipocytes, and being capable of inducing a reduction of body weight in an animal.

22. (Withdrawn) The immunogenic fragment of an ob polypeptide of Claim 21, which is selected from the group consisting of

Val-Pro-Ile-Gln-Lys-Val-Gln-Asp-Asp-Thr-Lys-Thr-Leu-Ile-Lys-Thr (SEQ ID NO:18);  
Leu-His-Pro-Ile-Leu-Ser-Leu-Ser-Lys-Met-Asp-Gln-Thr-Leu-Ala (SEQ ID NO:19);  
Ser-Lys-Ser-Cys-Ser-Leu-Pro-Gln-Thr-Ser-Gly-Leu-Gln-Lys-Pro-Glu-Ser-Leu-Asp  
(SEQ ID NO:20); and  
Ser-Arg-Leu-Gln-Gly-Ser-Leu-Gln-Asp-Ile-Leu-Gln-Gln-Leu-Asp-Val-Ser-Pro-Glu-Cys  
(SEQ ID NO:21).

Claims 23-27 (Canceled)

28. (Withdrawn) An antibody to the ob polypeptide of Claim 17.

29. (Withdrawn) An antibody to the ob polypeptide of Claim 18.

30. (Withdrawn) A method for preparing an antibody to an ob polypeptide, comprising

- A. conjugating the immunogenic fragment of an ob polypeptide of Claim 19 to a carrier protein;
- B. immunizing a host animal with the ob polypeptide fragment-carrier protein conjugate of step A admixed with an adjuvant; and
- C. obtaining antibody from the immunized host animal.

31. (Withdrawn) An antibody to an ob polypeptide prepared according to a method comprising:

- A. conjugating an immunogenic fragment of an ob polypeptide of Claim 19 to a carrier protein;
- B. immunizing a host animal with the ob polypeptide fragment-carrier protein conjugate of step A admixed with an adjuvant; and
- C. obtaining antibody from the immunized host animal.

32. (Withdrawn) The antibody of Claim 28, 29, or 31 comprising a polyclonal antibody.
33. (Withdrawn) The antibody of Claim 28, 29, or 30 comprising a monoclonal antibody.
34. (Withdrawn) An immortal cell line that produces a monoclonal antibody according to Claim 33.
35. (Withdrawn) The antibody of Claim 28, 29, or 31 labeled with a detectable label.
36. (Withdrawn) The antibody of Claim 35 wherein the label is selected from the group consisting of enzymes, chemicals which fluoresce, and radioactive elements.
37. (Withdrawn) A method for measuring the presence of an ob polypeptide in a sample, comprising:
- A. contacting a sample suspected of containing an ob polypeptide with an antibody that binds to the ob polypeptide under conditions which allow for the formation of reaction complexes comprising the antibody and the ob polypeptide,
  - B. detecting the formation of reaction complexes comprising the antibody and ob polypeptide in the sample;
- in which detection of the formation of reaction complexes indicates the presence of ob polypeptide in the sample.
38. (Withdrawn) The method of Claim 37 in which the antibody is bound to a solid phase support.
39. (Withdrawn) The method of Claim 38 which further comprises contacting the sample with a labelled ob polypeptide step (A), and removing unbound substances prior to step (B), and in which the formation of reaction complexes in the sample is detected by observing a decrease in the amount of labelled ob polypeptide in the sample.
40. (Withdrawn) The method of Claim 38 which further comprises contacting the sample with a

labelled antibody in step (A), which labelled antibody is an anti-ob polypeptide antibody, and removing unbound substances prior to step (B), and in which the formation of reaction complexes in the sample is detected by observing an increase in the amount of labelled antibody in the sample.

41. (Withdrawn) The method of Claim 37 in which an ob polypeptide is bound to a solid phase support.

42. (Withdrawn) The method of Claim 41 which further comprises contacting the sample with an ob polypeptide in step (A), and removing unbound substances prior to step (B), and in which the antibody is labelled and the formation of reaction complexes in the sample is detected by observing a decrease in the amount of labelled antibody.

43. (Withdrawn) A method for evaluating the level of ob polypeptide in a biological sample comprising

- A. detecting the formation of reaction complexes in a biological sample according to the method of Claim 30; and
- B. evaluating the amount of reaction complexes formed, which amount of reaction complexes corresponds to the level of ob polypeptide in the biological sample.

44. (Withdrawn) A method for detecting or diagnosing the presence of a disease associated with elevated or decreased levels of ob polypeptide in a mammalian subject comprising:

A. evaluating the level of ob polypeptide in a biological sample from a mammalian subject according to Claim 43; and

B. comparing the level detected in step (A) to a level of ob polypeptide present in normals or in the subject at an earlier time;

in which an increase in the level of ob polypeptide as compared to normal levels indicates a disease associated with elevated levels of ob polypeptide, and decreased level of ob polypeptide as compared to normal levels indicates a disease associated with decreased levels of ob polypeptide.

45. (Withdrawn) A method for monitoring a therapeutic treatment of a disease associated with elevated or decreased levels of ob polypeptide in a mammalian subject comprising evaluating the levels of ob polypeptide in a series of biological samples obtained at different time points from a mammalian subject undergoing a therapeutic treatment for a disease associated with elevated or decreased levels of ob polypeptide according to the method of Claim 43.

46. (Withdrawn) The method according to Claim 44 or 45, wherein the disease associated with elevated levels of ob polypeptide is selected from the group consisting of AIDS, cachexia, cancer, and anorexia nervosa.

47. (Withdrawn) The method according to Claim 44 or 45, wherein the disease associated with decreased levels of ob polypeptide is selected from the group consisting of obesity, Type II diabetes, hypertension, and elevated blood lipids.

48. (Withdrawn) A test kit for measuring the presence or amount of ob polypeptide in a sample, comprising:

- A. an anti-ob polypeptide antibody of Claim 28, 29, or 30;
- B. means for detecting binding of the anti-ob polypeptide antibody to ob polypeptide in a sample;
- C. other reagents; and
- D. directions for use of the kit.

49. (Withdrawn) A method for changing the body weight of a mammal comprising inhibiting the expression of an ob polypeptide encoded by a nucleic acid of Claim 2.

50. (Withdrawn) The method according to Claim 49 comprising expressing an antisense nucleic acid molecule hybridizable to a nucleic acid that expresses the ob polypeptide, expressing a ribozyme that cleaves a nucleic acid that expresses the ob polypeptide, administering an antisense nucleic acid molecule hybridizable to a nucleic acid that expresses the ob polypeptide, and administering a ribozyme that cleaves a nucleic acid that expresses the ob polypeptide

51. (Withdrawn) A pharmaceutical composition for reducing body weight of an animal comprising the ob polypeptide of Claim 17 and a pharmaceutically acceptable carrier.
52. (Withdrawn) A pharmaceutical composition for reducing body weight of an animal comprising the ob polypeptide of Claim 18 and a pharmaceutically acceptable carrier.
53. (Withdrawn) A method for reducing the body weight of an animal comprising administering an amount of a pharmaceutical composition of Claim 52 effective to reduce the body weight of an animal to an animal believed to be in need of decreased body weight.
54. (Withdrawn) The method according to Claim 53 wherein the animal is a human, and the ob polypeptide is human ob polypeptide.
55. (Withdrawn) A method for reducing the body weight of a mammal comprising increasing the expression of a protein encoded by the nucleic acid of Claim 2.
56. (Withdrawn) A pharmaceutical composition for increasing the body weight of an animal comprising an antagonist of an ob polypeptide
57. (Withdrawn) The pharmaceutical composition of Claim 56, wherein the antagonist is selected from the group consisting of an antibody that binds to and neutralizes the activity of ob polypeptide, a fragment of the ob polypeptide that binds to but does not activate the ob receptor, and a small molecule antagonist of the ob polypeptide.
58. (Withdrawn) A method for increasing the body weight of an animal comprising administering an amount of the pharmaceutical composition of Claim 56 effective to cause an increase in body weight to an animal believed to be in need of increased body weight.
59. (Currently amended) An isolated nucleic acid molecule selected from the group consisting of:

- a) a nucleic acid having the sequence of SEQ ID NO:1;
- b) a nucleic acid molecule having the sequence of SEQ ID NO:3;
- c) a nucleic acid molecule having the sequence of SEQ ID NO:22; and
- d) a nucleic acid ~~sequence~~ molecule that hybridizes under moderate stringency conditions to any one of the nucleic acids of (a), (b), and (c), ~~and~~
- e) ~~a nucleic acid sequence that encodes an expression product of an amino acid sequence encoded by any of the foregoing nucleic acid sequences.~~

60. (Canceled)

61. (Currently amended) An isolated nucleic acid molecule that encodes an OB polypeptide capable of modulating body weight; and having one or more polymers attached thereto, said nucleic acid optionally in a pharmaceutical carrier, wherein said OB polypeptide encoded by said isolated nucleic acid comprises the amino acid sequence set out in:

- a) SEQ ID NO:2;
- b) amino acids 22-167 of SEQ ID NO:2;
- c) SEQ ID NO:4 or
- d) amino acids 22-167 of SEQ ID NO:4.

62. (Currently amended) An isolated nucleic acid molecule that encodes an OB polypeptide capable of modulating body weight; and having one or more polymers attached thereto, said nucleic acid optionally in a pharmaceutical carrier, wherein said OB polypeptide encoded by said isolated nucleic acid comprises the amino acid sequence set out in

- a) SEQ ID NO:5;
- b) amino acids 22-166 of SEQ ID NO:5;
- c) SEQ ID NO:6 or
- d) amino acids 22-166 of SEQ ID NO:6.

63. (Currently amended) An isolated nucleic acid molecule that encodes an OB polypeptide capable of modulating body weight; and having one or more polymers attached thereto, said

nucleic acid optionally in a pharmaceutical carrier, wherein said OB polypeptide encoded by said isolated nucleic acid has 83 percent or greater amino acid sequence identity to the OB polypeptide amino acid sequence set out in SEQ ID NO:2, 4, 5 or 6.

64. (Currently amended) An isolated nucleic acid molecule that encodes an OB polypeptide, capable of modulating body weight, and having one or more polymers attached thereto, said nucleic acid optionally in a pharmaceutically acceptable carrier, wherein said OB polypeptide encoded by said isolated nucleic acid is an OB polypeptide variant comprising amino acids 22-167 of SEQ ID NO:4 in which one or more amino acids selected from the group consisting of amino acids 53, 56, 71, 85, 89, 92, 95, 98, 110, 118, 121, 122, 126, 127, 128, 129, 132, 139, 157, 156, 163 and 166, according to the numbering of SEQ ID NO: 4, is substituted with a conserved amino acid.

65. (Currently amended) An isolated nucleic acid molecule that encodes an isolated nucleic acid molecule that encodes an OB polypeptide, capable of modulating body weight, and having one or more polymers attached thereto, said nucleic acid optionally in a pharmaceutically acceptable carrier, wherein said OB polypeptide encoded by said isolated nucleic acid is an OB polypeptide variant comprising amino acids 22-167 of SEQ ID NO:4 in which one or more of amino acids selected from the group consisting of amino acids 53, 56, 71, 85, 89, 92, 95, 98, 110, 121, 122, 127, 128, 129, 139, 157, 159 and 163, according to the numbering of SEQ ID NO: 4, is substituted with the particular amino acid present at the corresponding position in SEQ ID NO: 2.

66. (Currently amended) An isolated nucleic acid molecule that encodes an OB polypeptide, capable of modulating body weight, and having one or more polymers attached thereto, said nucleic acid optionally in a pharmaceutically acceptable carrier, wherein said OB polypeptide encoded by said isolated nucleic acid is an OB polypeptide variant comprising amino acids 22-167 of SEQ ID NO:6 in which one or more of amino acids selected from the group consisting of amino acids 52, 55, 70, 84, 88, 91, 94, 97, 109, 117, 120, 121, 125, 126, 127, 128, 131, 138, 156, 158, 162 and 165, according to the numbering of SEQ ID NO: 6, is substituted with a conserved



amino acid.

67. (Currently amended) An isolated nucleic acid molecule that encodes an OB polypeptide, capable of modulating body weight; and having one or more polymers attached thereto, said nucleic acid optionally in a pharmaceutically acceptable carrier, wherein said OB polypeptide encoded by said isolated nucleic acid is an OB polypeptide variant comprising amino acid 22-167 of SEQ ID NO:6 in which one or more of amino acids selected from the group consisting of amino acids selected from the group consisting of amino acids 52, 55, 70, 84, 88, 91, 94, 97, 109, 120, 121, 125, 126, 127, 128, 138, 156, 158 and 162, according to the numbering of SEQ ID NO: 6, is substituted with the particular amino acid at the corresponding position in SEQ ID NO: 5.

68. (Canceled)

69. (Currently amended) The nucleic acid of any one of claims 59 to 67 ~~claim 68~~, wherein at least one of said polymers is a polyamino acid and is N-terminally attached to said OB polypeptide.

70. (Currently amended) The nucleic acid of any one of claims 59 to 67 ~~claim 68~~, wherein at least one of said polymers is a polyamino acid and is C-terminally attached to said OB polypeptide.

71. (Currently amended) The nucleic acid of any one of claims 59 to 70 67, wherein said nucleic acid is selected from the group consisting of DNA or RNA.

72. (Currently amended) The nucleic acid of any one of claims 59 to ~~70~~ 67, wherein said nucleic acid is detectably labeled.

73. (Currently amended) A cloning vector comprising a nucleic acid of any one of claims 59 to ~~70~~ 67.

74. (Currently amended) An expression construct comprising a nucleic acid molecule of any one of claims 59 to ~~70~~ 67 operatively associated with an expression control sequence.

75. (Previously presented) The expression vector of claim 74, wherein said expression control sequence is selected from the group consisting of cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 or adenovirus, the lac system, the trp system, the TAC system, the major operator and promoter regions of phage  $\lambda$ , the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast  $\alpha$ -mating factors.

76. (Previously presented) A unicellular host transfected with a cloning vector of claim 73.

77. (Previously presented) A host cell transformed with an expression construct of claim 74.

78. (Previously presented) The host cell of claim 77, wherein said host cell is selected from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, Pichia yeasts, CHO, R1.1, B-W, L-M, COS-1, COS-7, BSC1, BSC40, BMT10 and cells, plant cells, insect cells and human cells in tissue culture.

79. (Currently amended) A method for preparing an OB polypeptide comprising;

- a) culturing a host cell of claim 76 ~~or 77~~ under conditions that allow the expression of said OB polypeptide; and
- b) recovering the expressed OB polypeptide.

80. (Previously presented) The method of claim 79, wherein said host cell is a bacterial cell.

81. (Previously presented) The method of claim 79, wherein the host cell is a yeast cell.

82. (Previously presented) The method of claim 79, further comprising:

- c) chromatographing the polypeptide on a Ni-chelation column; and

d) purifying the polypeptide by gel filtration.

83. (Previously presented) The method of claim 82, further comprising after step (c) and prior to step (d), chromatographing the OB polypeptide on a strong cation exchanger column.

84. (NEW) A method for preparing an OB polypeptide comprising:

- a) culturing a host cell of claim 77 under conditions that allow the expression of said OB polypeptide; and
- b) recovering the expressed OB polypeptide.

85. (NEW) The method of claim 84, wherein said host cell is a bacterial cell.

86. (NEW) The method of claim 84, wherein the host cell is a yeast cell.

87. (NEW) The method of claim 84, further comprising:

- (c) chromatographing the polypeptide on a Ni-chelation column; and
- (d) purifying the polypeptide by gel filtration.

88. (NEW) The method of claim 86, further comprising after step (c) and prior to step (d), chromatographing the OB polypeptide on a strong cation exchanger column.